

THE LEUCOCYTES OF ARBACIA PUNCTULATA

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Geddes (1880), whose studies supersede earlier investigations, distinguished two main types of leucocytes¹ in sea-urchins: colorless, hyaline or granular elements and colored ones with the pigment attached to cytoplasmic inclusions. He found the first to be concerned with clotting and with phagocytosis of foreign matter, observations repeatedly confirmed later; the second were surmised to serve respiration. Cuenot (1891) observed that certain granulocytes of sea-urchins liberate their albuminoid or fatty inclusions throughout the organism and claimed that these granulocytes develop from the hyaline elements (or within them), as the latter take up nutrient matter from the digestive system and transform it into the cytoplasmic granules. His later studies (1897) brought him to entirely different conclusions, viz.: that the cytoplasmic inclusions represent end products of metabolism, absorbed and eliminated by these cells, a view also held by Delage and Herouard (1913). Kindred (1924, 1926), however, supports the earlier views of Cuenot. The close connection of the granular leucocytes with the digestive tract and their penetration into its lumen through the intestinal epithelium is interpreted as a secretory function by Frenzel (1892).

Saint-Hilaire (1897) drew a sharp line between the agranular phagocytes, which he observed to ingest foreign matter and dead cells, and the granulocytes. He confirmed earlier observations regarding the abundance of the latter in and around the intestinal wall but found no indication that they are concerned with nutritive processes. A search for granulocytes in the gonads revealed no great numbers in them and no clue as to their function, leading him to the then prevailing notion that they help eliminate waste products.

The red pigment of the granulocytes, named echinochrome by MacMunn (1885), is claimed by him to be an oxygen carrier, a view shared by Griffiths (1892). Canan (1927) found it to be an activator rather than a carrier of oxygen. Crude extracts of echinochrome are claimed by Friedheim (1932) to increase the respiratory rate in sea-urchin eggs; this, however, is denied by Tyler (1939) for the chemically pure substance. Echinochrome was also found to be present in the eggs and test of *Arbacia punctulata* (McClendon, 1912).

Comparative hematological studies on representatives of tunicates, insects, annelids and the coelenterate *Tubularia* (Liebman, 1946, 1947 *et ante*), have revealed two main types of leucocytes in these organisms: ameboid, agranular *phagocytes*, and non- or poorly ameboid, granular elements having a nutritive function

¹ This term is applied to all blood cells excluding those containing hemoglobin: the erythrocytes. "Granulocytes" designates elements with endogenous, regularly occurring cytoplasmic inclusions, irrespective of their size.

and hence termed by the writer *trephocytes*. Each kind was found to exhibit distinctive morphological and physiological characteristics, having frequently a separate locus of origin. The present investigation disclosed that conditions in *Arbacia* do not differ fundamentally from those found in other invertebrates studied and thus an extension of the scheme outlined above is permitted.

MATERIAL AND METHODS

Specimens for cytological studies were collected and preserved at the Woods Hole Marine Biological Laboratory during June and July, 1945. Additional material was secured and observations in the living state were made at the end of July, 1948. All were performed on fully grown, apparently healthy, individuals.

The leucocytes of *Arbacia* undergo profound changes during fixation and in some cases disintegrate beyond recognition. In addition, the pigments which characterize the various kinds of trephocytes dissolve in the fixatives and usually attach themselves to non-colored or differently colored elements, which easily leads to further confusion and misinterpretations. To avoid these pitfalls, observations on living leucocytes were made the basis of the present investigation. The blood (perivisceral fluid) was consequently studied extensively in the living condition shortly after removal, and also in hanging drop cultures kept at room temperature.

Blood-smears prepared in the conventional way proved of little value. The only method giving satisfactory results was a modification of the formalin-vapor technique described earlier (Liebman, 1945a): the blood is spread in a thick layer on a slide and placed in a moist chamber, a Petri-dish lined with wet filter-paper serving well the purpose. After 30 minutes the cover of the dish is slightly lifted and a wad of cotton soaked with formalin placed near, but not in contact with, the slide. Fifteen or twenty minutes later the slide is removed, air-dried and stained. The leucocytes, which become active in the moist chamber, are fixed by this method in the characteristic forms as they appear in life. Hanging drop cultures were also successfully fixed by this method. Preparations from blood, quickly withdrawn from the body and injected directly into the fixative, proved useful for some purposes since this method fixes certain elements in the same condition as they are found within the organism; it is referred to in the text as "whole-blood" method.

Preparations showing leucocytes fixed *in situ* are preferable where nuclear structures have to be considered. Aside from pigment displacement, mentioned earlier, the leucocytes—especially the granular kind—usually appear here in a contracted, more or less spherical shape, which they seldom assume in life; this, however, proved of considerable advantage when measurements had to be made and these were performed on said material. In fixing tissues or whole organisms, Zenker-formol produced the best results and was used extensively, except when other considerations required different methods.

Wright's blood-stain, strongly diluted in distilled water, and Mallory's connective tissue stain gave the most satisfactory results. Mallory's stain produced particularly sharp pictures of the minute nuclear and cytoplasmic structures, as well as a differential staining of the trephocytes after Zenker-formol fixation.

I am greatly indebted to Dr. Ross F. Nigrelli for his generous help and advice in preparing the photomicrographs.

OBSERVATIONS

A. The trephocytes

Calculations based on a count of 1608 cells from the perivisceral fluid of four individuals showed 50.4 per cent to be trephocytes, the remainder phagocytes. However, the number of trephocytes in the whole organism was probably higher, as they were practically the only leucocytes encountered in the ovaries; reports also agree that they are predominant in the digestive tract.

Observations *in vitro* revealed three kinds of trephocytes, having respectively green, red or colorless cytoplasmic inclusions. For sake of brevity they are referred to here as green, red or colorless trephocytes.

The green trephocytes. These are the least numerous, constituting 10.2 per cent of all trephocytes. They are the smallest in size, with an average diameter of $7\ \mu$ and a range of $6-8\ \mu$. The nucleus is small, round and lacks a nucleolus but,

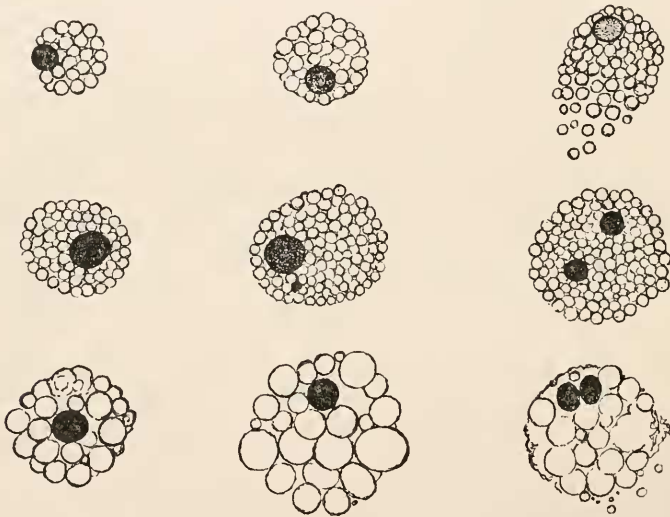


FIGURE 1. Trephocytes from an animal fixed in toto; upper row: green variant; median row: red variety; lower row: colorless variety. Camera lucida drawing; $1650\times$. In each row the element at left represents a young form, that at right an old one.

in contrast to the two other kinds, it shows minute chromatin granules (Fig. 1, first row). The cytoplasmic inclusions are fine, rather uniform in size and of a lemon-green color in the living cell. They are moderately eosinophilic or, in a small proportion of cases, chromophobic. With Mallory the granules stain reddish-orange, as does the nucleus.

In the living state, the green trephocytes occasionally contain a number of red cytoplasmic granules like those found in the next variant.

The red trephocytes (Fig. 1, second row) were the most numerous, comprising 48 per cent of the group's total. They average $9.2\ \mu$ in diameter with a range of $6.7-12\ \mu$. Their nucleolar nucleus is somewhat larger than that of the green kind, occasionally dumb-bell shaped or fragmented into two portions. When stained, it

assumes a flat, structureless aspect. The cytoplasmic granules are uniform in appearance and of the same size as those previously mentioned, but often coarser in the largest elements. In life they are of a brownish-red color due to the presence of echinochrome. They stain light-green with Wright and a brownish-yellow or light blue with Mallory.

The *colorless trephocytes* comprised 41.8 per cent of the total during the period of investigation. They are the largest kind (Fig. 1, third row), averaging $10.2\ \mu$ in diameter with extremes ranging from 6.0 – $13.4\ \mu$. The nucleus is minute, often smaller than that in the green kind, anucleolar and devoid of structures; in the larger cells it is frequently pycnotic, fragmented or occasionally missing, having apparently dissolved in the cytoplasm. The cytoplasmic inclusions are colorless or faintly greenish in life, irregular in size but predominantly very coarse. They are very strongly basophilic, occasionally slightly metachromatic; Wright's stain makes them appear almost black and completely opaque. With Mallory they stain a light-blue.

Chemical nature of the inclusions. The difficulties encountered in the cytological study of the trephocytes apply also to the cytochemical approach, hence our examinations should be considered of a preliminary character and the information gained rather of a tentative nature. Definite conclusions could only be drawn after a special study of the problem, with new or specifically adapted methods.

Routine techniques did not reveal the presence of osmio- or sudanophilic matter in any of the trephocytes. Orange-yellow coloration after Mallory's staining, assumed by some authors to suggest phospholipins, appeared in the green, red and occasionally also in the colorless inclusions. Glycogen, as indicated by Langhans' iodine-method and Best's staining, and checked with the sputum test, was only very rarely found. The strong basophilia of the colorless inclusions suggests the presence of ribonucleic acid and their positive reaction towards mucicarmine—a mucin. The color of the inclusions of the red trephocytes is generally attributed to echinochrome.

Common characteristics of the trephocytes. The regular presence of endogenous granules is *per se* a common feature of the trephocytes. Another is the characteristic nucleus.

Additional features peculiar to all trephocytes are their function, to be discussed later, and the lack of phagocytic capacity. Observations *in vitro*, both on freshly drawn blood as well as on cultures, in no case revealed ingestion of foreign matter, although in the older cultures extensive phagocytosis was evident in other cells. Similarly, in the fixed material no indication of phagocytosis on the part of the trephocytes was found.

Another common peculiarity of all trephocytes is their way of locomotion. They move by emitting blunt lobopodes into which the granules follow quickly. When moving, the cells usually assume an oblong appearance, frequently with a trail of granules or cytoplasm in their wake (Fig. 2), which gives them a striking resemblance to rat-tailed maggots.

As against these common features, the composition of the granules thus appears to be the main factor distinguishing the three kinds of trephocytes. Even here, however, the limits do not appear to be fast and absolute; this is suggested by some tinctorial as well as cytochemical characteristics common to all granules.

Genetic relationship of the trephocytes. The relatively well differentiated nucleus, the small size and the rather low frequency of senile, disintegrating forms, suggest that the green trephocytes are the youngest of the three variants. Of the green trephocytes, the chromaphobe forms are the smallest, have the largest and best

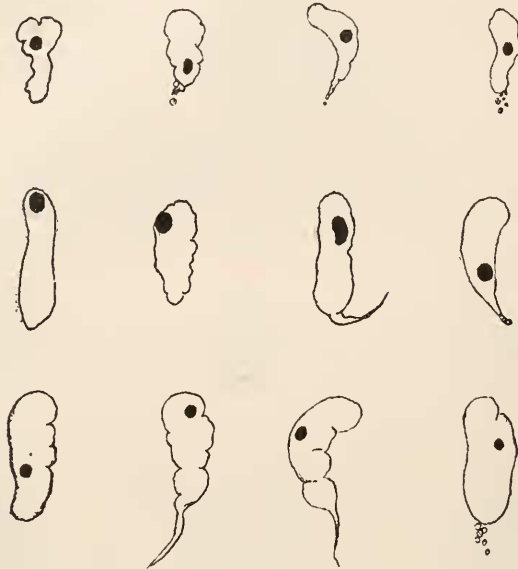


FIGURE 2. Outlines of living trephocytes in motion; from a hanging drop culture; camera lucida; 1000 $\times$. Upper series: green; middle series: red; lower series: colorless trephocytes.

differentiated nuclei and are consequently regarded as the youngest in this group. Also, chromophobia of the inclusions is peculiar to very young cells and has repeatedly been observed by the writer in the earliest developmental stages of granulocytes (trephocytes of annelids, eosinophils of fishes and amphibians). The largest forms amongst the green trephocytes, on the other hand, generally represent the

PLATE I

All figures are unretouched photomicrographs of sections of ovaries fixed in Zenker-formol and stained with Mallory's connective tissue mixture.

FIGURE 1. Cross-section from an ovary, showing the interovular spaces filled with disintegrated trephocytes. The clear areas around the oöcytes are due to shrinkage; 600 $\times$.

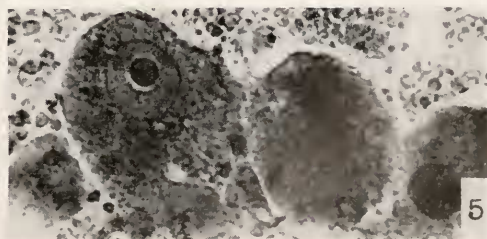
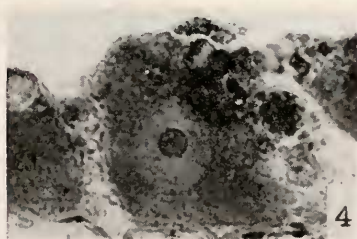
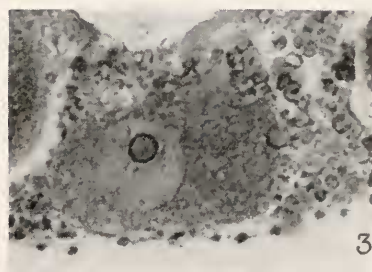
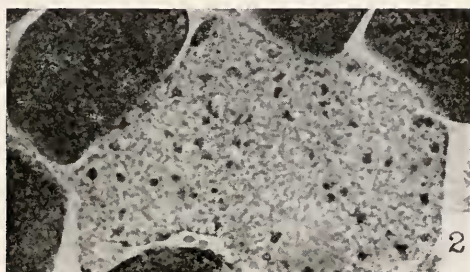
FIGURE 2. A compact mass of partly disintegrated red trephocytes surrounded by oöcytes; the dark spots in the mass represent nuclei; 450 $\times$.

FIGURE 3. An oöcyte showing uptake of trephocytic granules along its free (upper) side; 650 $\times$; note lack of cell-wall and continuous stream of granules at the ingesting site. In this, as in the following two figures, the lower edge of the picture corresponds to the site of attachment of the oöcytes to the ovarian wall.

FIGURE 4. A young oöcyte in the process of uptake of larger droplets and a nucleus (along the upper right side); 800 $\times$.

FIGURE 5. Two oöcytes engorged with trephocytic granules; 800 $\times$. A close inspection shows small perforations in the ovular walls.

PLATE I



oldest elements, showing most frequently pycnotic nuclei and signs of cellular disintegration. This scheme, if not too rigidly applied, holds also for the two other variants, the smallest usually representing the youngest, the largest the most mature individuals of each group. Among the three groups, the colorless trephocytes show the most pronounced signs of maturity and disintegration.

Elements which show green and red inclusions are not infrequent, indicating the probable origin of the red variant from the green one. The colorless forms often exhibit a more or less green hue, suggesting also a possible development from the green kind. Tinctorially, on the other hand, the colorless and red granules occasionally show common characteristics. Despite extensive search, however, we have not found in the perivisceral fluid colorless elements with clear-cut transitional or mixed characteristics, as is the case with the trephocytes containing green and red granules. This does not preclude the possibility that the transformation into colorless elements takes place in some organ.

Neither in the blood nor in the tissues studied (gonads and adjoining tissues) were there any indications suggesting development of the trephocytes from phagocytes, nor was there any indication of a common origin.

The function of the trephocytes. Distintegration of all three variants, accompanied by a scattering of the cellular components was observed both *in vitro* as well as in fixed material, in the blood as well as in tissues. This is usually preceded or paralleled by loss of staining capacity of the nucleus or, more rarely, by its becoming pycnotic. Occasionally the cell components simply fuse into one or several droplets; this was observed mostly in the colorless trephocytes. Considering the ubiquity and the high frequency of the processes described, they must be considered as normal phenomena.

The ultimate fate of the trephocytes and their derivatives, as well as the nature of their inclusions, suggest a trophic function. This is particularly evident from their relation to the growing eggs and to the phagocytes, both objects of close study in the present investigation.

The ovaries were found to be densely packed with trephocytes, most of them in a partly or fully disintegrated state. This cellular brei surrounds the oöcytes from all sides except where they border the ovarian wall, as is the case in the youngest elements (Plate I, Fig. 1). It appears to be well attached to the ovules, as witnessed by the difficulty of separating it from the oöcytes in isolates. In some places the trephocyte masses form extensive, uninterrupted islands in the ovary (Plate I, Fig. 2).

Only the red and colorless variants of trephocytes were found in the ovaries. Because of their disintegrated state, it was impossible to submit them to a differential count, the general impression being, however, that their proportion roughly corresponds to that in the blood.

Suitable preparations reveal that the young oöcytes take up granules and droplets of the cellular brei surrounding them. This apparently takes place to a great extent by a process of fusion: the ovular membrane in the areas concerned is very thin, discontinuous or missing and the granules form an uninterrupted stream from the exterior practically up to the nucleus (Plate I, Fig. 3). Occasionally large droplets, some still showing unmistakable marks of nuclei, are found to be taken up in this manner by the oöcytes (Plate I, Fig. 4). On the other hand, very small

granules appear to penetrate through the ovular membranes and are frequently found sticking in them (Plate I, Fig. 5). Extremely small perforations are also detectable in the wall of the young oöcytes, as shown in the same figure, but these are not necessarily preformed openings but may represent empty spaces from which granules have been dissolved in the process of fixation, dehydration, etc. The result of all these activities is that the young oöcytes frequently appear gorged with granules (Plate I, Fig. 5), whose physical and tinctorial appearance leaves little doubt regarding their origin from the trephocytes.

The phenomena described indicate that a considerable proportion of the egg's substance consists of material taken up in corpuscular form and derived from the trephocytes. Since a large fraction of this material can be traced to their red variety, the possibility should not be precluded that the echinochrome found in the eggs of *Arbacia* also has its origin in the trephocytes.

B. *The phagocytes*

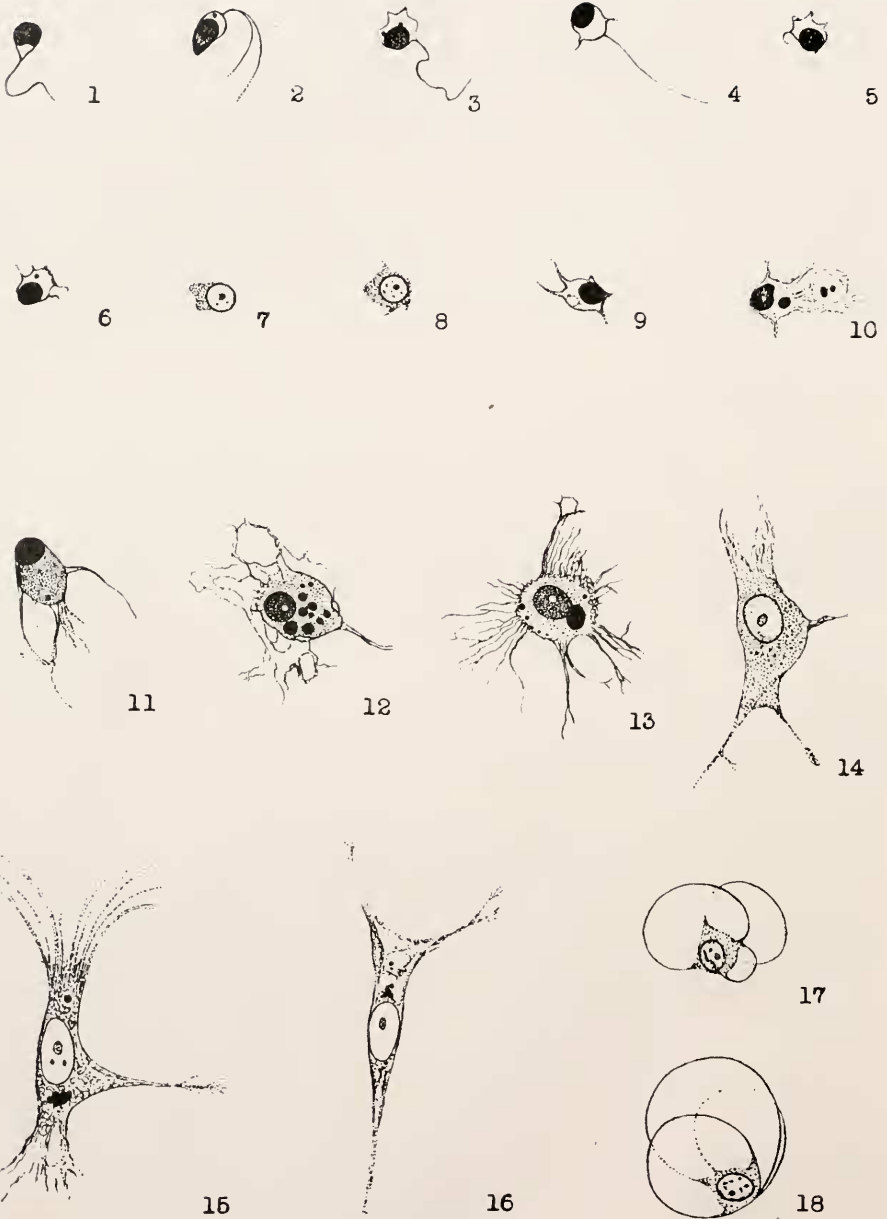
The variety of these elements in the species studied, as well as in the echinoderms in general, has hardly its equal in the animal kingdom. Still, even more clearly than in the case of trephocytes, the present studies reveal that they are merely developmental and functional stages of the same cell, some possibly representing reversible adaptations to the medium in which they are found. They are here classified into four groups, according to their outstanding characteristics: (1) Flagellated forms, (2) Ameboid forms, (3) Fibroblasts and (4) Petaloid forms.

The flagellated phagocytes represent the smallest and apparently youngest stages of this group (Table II, Fig. 1-4). In the living state they appear as spherical elements gyrating and moving briskly among the other blood cells. When fixed, they show one—rarely two—flagella and a spherical, ovoid or piriform cell-body. The nucleus is relatively large and nucleolated, the chromatin fine. (In smears, the nucleolus of these, as well as of other small elements, is rarely visible.) Both *in vitro* as well as in fixed preparations the cytoplasm occasionally shows trephocytic granules, suggesting a certain phagocytic capacity.

Flagellated elements are frequently observed *in vitro* to settle, lose their flagellum and become ameboid. Figures 3, 4 and 5-8 (Plate II) represent such cells prior to and soon after this transition. Except for the absence of the flagellum and the regular presence of small pseudopods, the elements, soon after settling, hardly differ from the flagellated forms. The small size, high nuclear-cytoplasmic ratio, and poor phagocytic capacity, as well as some other characteristics, suggest a similarity of the flagellated and young ameboid forms to the vertebrate lymphocytes.

The ameboid phagocytes. Between the smallest, aflagellar, ameboid forms and the largest elements—the macrophages—innumerable transitions can be observed both *in vitro* and in the fixed preparations (Plate II, Figs. 5-13). They are all characterized by a relatively large nucleus of a round, spheroid, occasionally bean-shaped form, and fine chromatin. A large nucleolus, sometimes two or three, is always present. The hyaline cytoplasm forms long slender pseudopods continuously changing shape and configuration. This ameboid capacity seems to increase with the size of the cells, the latter again being, in general, an expression of their physiological age. Small and medium-sized forms were most commonly encountered in the blood during the present investigation. The whole group cor-

PLATE II



Various forms of phagocytes; camera lucida drawings; 1300 $\times$. Figures 1-6 and 9-16 from a formalin-vapor preparation. Figures 7, 8 from an animal fixed *in toto*; Figures 17, 18 from a whole-blood preparation. The black matter in the cytoplasm represents ingested trephocyte material.

responds to the vertebrate mononuclears; the largest forms show in most respects a similarity to the macrophages of vertebrates and other invertebrates.

The fibroblasts. In hanging drop cultures, elements closely resembling vertebrate fibroblasts (Plate II, Figs. 15, 16) appeared within 24 hours. Transitional forms between them and the macrophages were observed both *in vitro* and in fixed material (Plate II, Fig. 14) and it is highly probable that they develop from macrophages. Without implying that they correspond in all details to their vertebrate counterparts, their general appearance unmistakably justifies their being classified as fibroblasts. The nucleus, except for being elongated, is similar to that of the macrophages; the cytoplasm is fibrillar or reticular, a characteristic appearing also in the macrophages in regions where long pseudopods are formed.

The petaloid phagocytes. Another variety of phagocytes is illustrated in Figures 17, 18 (Plate II). Their ectoplasm forms several roughly semicircular membranes or flaps which continuously change shape and position. The nucleus of this form does not differ from that of the ameboid phagocytes. Similar phagocytes have been described in earthworms where their membranes are oblong and arranged around the cell somewhat like petals.

Observations on freshly-drawn blood as well as on whole-blood preparations indicate that this form is very common in the perivisceral fluid. *In vitro* it was only found in a suspended condition and observations lead to the conclusion that it should be considered an adaptation to a floating way of existence, the membranes—apart from other functions—acting to increase the buoyancy, as in the case of planktons. When the cell settles on a substrate it changes into an ameboid form, the membranes often persisting for a considerable time. A similar change was observed in the phagocytes of earthworms, when they come in contact with a solid surface (Liebman, unpublished observations) and in human leucocytes (Bessis and Bricka, 1949). There are indications that the ameboid form of *Arbacia*, if detached from the substrate, turns into the floating variety.

Common characteristics of the phagocytes. The nucleus is single, relatively large and never missing; its form more or less spherical, ellipsoid or bean-shaped. A large nucleolus, occasionally accompanied by one or two smaller ones, is always present.

The cytoplasm is hyaline and devoid of endogeneous inclusions. Ameboid movement appears in all forms in the presence of a substrate and increases markedly with the volume of the cytoplasm. Phagocytosis is present in all stages.

Function of the phagocytes. Though the phagocytes are known to be involved in several other functions, the present study is solely concerned with their capacity to take up formed matter. Under natural conditions this, except for occasional bacteria, consists almost exclusively of trephocytic granules or whole cells.

The flagellated forms were occasionally found to contain trephocyte granules of the green, red, or colorless variety in their cytoplasm. Because of their rapid movements, it could not be ascertained whether these are taken up by pseudopods or with the aid of the flagellum.

The ameboid forms phagocytose in the familiar way of engulfment with the aid of pseudopods. Generally, their phagocytic capacity appears to increase with the size of the cell or, more correctly, with the amount of the cytoplasm. In the fibroblasts, on the other hand, despite their relatively large size, the phagocytic capacity appears to be considerably reduced.

The petaloid forms show the highest phagocytic activity. This, however, is not necessarily due to an inherent quality but is probably owing to the ability to operate unimpeded in all three dimensions, in contrast to corresponding ameboid variants, phagocytosing on a substrate.

Whole, living trephocytes were repeatedly observed *in vitro* to be attacked by single, occasionally several, petaloid phagocytes. The trephocytes are caught by the flaps moving their surfaces close to each other and finally over the prey, somewhat in the manner in which a ball is caught in two hands. The engulfed trephocyte is seen moving vigorously within the phagocyte for a considerable time but ultimately undergoes gradual digestion, though its movements occasionally help it to "escape." This peculiar form of phagocytosis is also common within the organism, and whole-blood and *toto* preparations show a relatively high percentage of phagocytes containing intact trephocytes or some in various degrees of digestion (Fig. 3).



FIGURE 3. Phagocytes with engulfed intact and digested trephocytes; from whole-blood preparations; camera lucida; 1650 $\times$.

The uptake of trephocytic matter and the engulfment and digestion of living elements cannot be considered as phagocytosis in the accepted sense, which usually implies ingestion of noxious substances to be eliminated from the organism. It represents a normal way of nutrition for the phagocytes, and the phagocytosis of live trephocytes—cannibalism.² This, of course, should not imply that the phagocytes do not ingest matter destined for elimination, like dead cells, bacteria, or introduced foreign substances.

DISCUSSION

Previous studies on trephocytes (Liebman, 1946, 1947) have revealed that formed matter liberated by them is taken up by a variety of cells: oöcytes, phagocytes and epithelial cells. This led to the conclusion that, in addition to chemical substances, the animal cell depends also on biological elements for nutrition.

The present, as well as some earlier observations, impel us to extend this notion further, by putting forward the idea that cannibalism apparently represents a normal process of cellular nutrition.

Uptake and digestion of whole trephocytes by the growing ovarian eggs are apparently common among invertebrates. In some cases (*Tubularia*) the trephocytes

² In the few instances where this term has been applied to cells, it is used both in a strict sense to denote ingestion of living elements by their own kind, as well as in cases where the phagocytosed cells are merely of the same organism. We propose to apply this term in the latter sense to all cases where a cell ingests and digests any other living cell of the same organism.

taken up by the egg are dead, thus excluding cellular cannibalism as understood by cytologists. In several tunicates investigated, however, as well as in some annelids (Liebman, *ibid.*), the trephocytes within the egg appear to be alive. In the tunicates, they seem to persist in this state for a considerable time—as within the phagocytes of *Arbacia*—before being digested.

In the vertebrates, lymphocytes have been reported from epithelial cells of the intestinal and renal tract of fishes and Amphibia (Loreti, 1934, 1935), the oöcytes and epidermal cells of amphibians (Liebman, 1945b, 1947), and from the epithelial cells of the digestive and respiratory tract of mammals (Andrew and assoc., 1945, 1946, 1947). Not in all these cases are the lymphocytes alive when entering other elements nor are they all digested by the host cells, but there are clear indications that cannibalism in some of these instances is not infrequent.

It is possible that Cuenot (1891) saw elements corresponding to trephocytes within hyaline cells (phagocytes) but, failing to observe digestion, assumed that the former develop within the latter.

The presence of living cells within others has been definitely established in tissue cultures. Lewis (1925) saw frog-mononuclears and epitheloid cells from tubercular rabbits engulf their own kind, but stresses the great rarity of such cases and their probable pathologic nature. Fischer and Dolschansky (1929) repeatedly observed small cells wandering in and out of stroma cells of spleen cultures. They have not observed digestion of these elements, which they consider as derivatives of the stroma cells. Some of their photographs (e.g., Figs. 5, 6) indicate however, that these wandering cells are lymphocytes and their often fragmented and pycnotic condition does not exclude the possibility that they are occasionally digested by their hosts.

The inclusions of the trephocytes are frequently colored, occasionally also the cytoplasm. Except for echinochrome, however, our knowledge of their colorants is extremely limited and even the precise role of the former in respiration, is not fully clarified.

Observations on the invertebrate erythrocytes strongly suggest that they represent a special kind of colored trephocytes. It was pointed out earlier (Liebman, 1946) that the erythrocytes of the polychaete *Cirratulus grandis* contain numerous lipid inclusions in the cytoplasm and a typical trephocytic nucleus while their fixed shape, cytoplasmic structure and presence of hemoglobin are characteristic of erythrocytes. Conditions in other polychaetes (Romieu, 1923), as well as in invertebrates generally (Ohuye, 1937), reveal similar traits in their erythrocytes. This similarity extends to the vertebrates where the first embryonic (primitive) erythrocytes contain nutritive inclusions in the cytoplasm (Maximow, 1927), and often exhibit a striking resemblance to trephocytes (Cameron, 1941, and others). Loss of the nucleus, characteristic of the mammalian erythrocytes, occasionally takes place in invertebrate red corpuscles and is a recurrent feature of the trephocytes proper. It is significant that this trait is common only to the erythrocytes and trephocytes among the blood cells.

The presence of a flagellum in the youngest stages of the phagocytes is probably due to their origin from the coelomic epithelium which is usually ciliated in the echinoderms.

Petaloid phagocytes are quite common among invertebrates. Goodrich (1920)

claims that the fine pseudopodes of the ameboid forms are either lateral views of the petals or artifacts, insisting that the only true form is the petaloid (his "membraneous") pseudopod. He also claims that the "petals" are merely folds of a continuous membrane. His extreme views are not borne out by our observations on *Arbacia* and earthworms.

Cuenot (1891) surmises that the movements of the flagellated forms prevent clotting of the blood. Coagulation of blood and the occlusion of wounds are generally agreed to be effected by the ameboid phagocytes forming a dense network with their fused pseudopods (pseudo-agglutination). Phagocytosis of introduced bacteria has been observed by Cernovodeanu and Henri (1906), and that of non-deposited sex products by Caullery and Siedlecki (1903).

SUMMARY

This investigation revealed the presence of two different types of leucocytes in *Arbacia*: trephocytes and phagocytes. An outline of their morphology, development and physiology is presented. No genetic relationship between the two kinds could be established, a circumstance which further tends to prove the distinctive nature of each type.

Particulate matter derived from the trephocytes and essentially of a nutritive nature, is taken up and incorporated by the growing oöcytes. A considerable fraction of this material has its origin in the red trephocytes which contain echinochrome. It is suggested that the echinochrome found in the eggs of the species investigated may also be derived from the trephocytes.

The occurrence of respiratory pigments among invertebrate trephocytes is discussed. It is pointed out that the erythrocytes of invertebrates, as well as the early stages of those of vertebrates, exhibit characteristics of trephocytes. The idea is advanced that the erythrocytes represent a special kind of trephocytes.

Formed trephocytic material was seen to be engulfed and digested by the phagocytes of *Arbacia*. This, as in the case of the oöcytes, is regarded as a normal process of cellular nutrition and lends further support to the thesis, expressed earlier by the writer, that the animal cell takes up and assimilates particulate cellular material in addition to substances in solution.

The engulfment and digestion of living trephocytes is described and discussed and additional cases of cellular cannibalism among invertebrates and vertebrates brought forward. The conclusion is reached that cannibalism is a normal and apparently fairly common form of nutrition of the animal cell.

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